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Antifungal Activity from 14-Helical β-Peptides

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Treating fungal infections represents a profound medical challenge. Natural and synthetic small molecule antifungal agents are known, but their use as therapeutics is limited by their inherent toxicity to humans and increasing incidence of resistance.[1] Host-defense peptides, components of the innate immune system that are very effective against prokaryotic pathogens, have been reported to display antifungal activity under some conditions in vitro,[2] but the relevance of these results to in vivo activity is unclear. Shai et al. have shown that hydrophobic appendages can enhance the antifungal activity of host-defense peptides and designed sequences.[3]

Several groups have explored unnatural oligomers composed of β-amino acids ("β-peptides") as mimics of host-defense α-peptides in the antibacterial context.[4] β-Peptide "foldamers" can be designed to adopt helical conformations that display discrete hydrophobic and cationic surfaces,[4] thereby mimicking the globally amphiphilic α-helical conformations of many host-defense peptides, including magainins and cecropins.[2] Some β-peptides display antibacterial activity comparable to that of the host-defense α-peptide prototypes.[4] Here we show that properly designed β-peptides function as antifungal agents under conditions that render host-defense α-peptides inactive against fungal pathogens.

We focused on *Candida albicans*, the most prevalent fungal pathogen in humans.[5] Minimum inhibitory concentrations (MIC) were evaluated with three *C. albicans* strains using procedures suggested by the Clinical and Laboratory Standards Institute (formerly known as the National Committee for Clinical Laboratory Standards, NCCLS)[6] (Table 1); little difference was observed among the three strains. The minimum fungicidal concentrations (MFC) for these *C. albicans* strains were assessed using a colony-forming assay. In every case the MFC was equivalent to the MIC (data not shown).

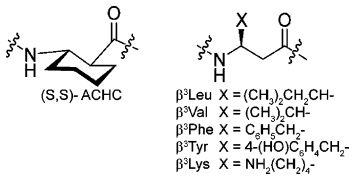
We evaluated two α-peptide sequences to gauge the antifungal activities of amphiphilic host-defense peptides in the NCCLS assay, which is performed at pH 7 and physiological ionic strength. Previous reports indicate that the activities of cecropins and magainins against *C. albicans* depend sensitively on assay conditions. At low ionic strength, such α-peptides inhibited growth above 10 μg/mL at pH 5.5, but were relatively inactive at pH 7.4.[7] At pH 7, magainin 2 reduced *C. albicans* growth by 50% at concentrations below 1 μg/mL in low ionic strength solutions but was inactive at physiologic ionic strength.[8] We find that neither cecropin B nor a magainin derivative displays any activity under the NCCLS assay conditions (the latter peptide, a triple mutant of magainin 2, has been widely employed because of its enhanced antibacterial activity relative to magainin 2 itself[9]). To determine whether the α-peptide inactivity we observed arises from proteolytic degradation, we examined the enantiomer of the magainin derivative. Enantiomeric host-defense peptides retain the antibacterial activity of their natural antipodes,[10] presumably because the mechanism of action involves bacterial membrane disruption rather than binding to a specific bacterial protein. The enantiomeric magainin derivative should resist protease attack, but this peptide is inactive in the NCCLS antifungal susceptibility assay. Thus, the lack of antifungal activity observed for the α-peptides does not result simply from proteolytic degradation.

Table 1. Average Minimum Inhibitory Concentrations (MIC) against *C. albicans* and Percent Hemolysis at the Average MIC

peptide	average MIC[a] μg/mL	% hemolysis at MIC[b]
L-magainin 2 derivative	>128	48*
D-magainin 2 derivative	>128	48*
β^3Tyr-(ACHC-β^3Val-β^3Lys)$_3$ (**1**)	16	21
β^3Tyr-(ACHC-β^3Leu-β^3Lys)$_3$ (**2**)	11	73
β^3Tyr-(ACHC-β^3Phe-β^3Lys)$_3$ (**3**)	8	80
β^3Tyr-(β^3Val-β^3Val-β^3Lys)$_3$ (**4**)	69	9
β^3Tyr-(ACHC-ACHC-β^3Lys)$_3$ (**5**)	21	7
(ACHC-β^3Val-β^3Lys)$_3$ (**6**)	17	5
β^3Lys-β^3Val-β^3Val-ACHC-β^3Lys-β^3Val-ACHC-ACHC-β^3Lys (**7**)	>128	ND

[a] Average of all MIC values obtained for three *C. albicans* strains (SC5314, ATCC 24433, and ATCC 90028). [b] For peptides with MIC higher than the highest concentration tested (128 μg/mL), the % hemolysis at 128 μg/mL is given and marked by an asterisk (*). ND indicates no hemolysis data were obtained.

We examined β-peptides intended to adopt 14-helical secondary structure (defined by 14-membered ring H-bonds formed between backbone C=O(i) and H−N(i-2) groups), because relatively short foldamers in this helical class have previously been shown to display potent antibacterial activity and low hemolytic activity.[4c,g] Since the mechanism of antimicrobial activity appears to involve membrane disruption, it is critical to examine the susceptibility of host cell membranes, for example, from human red blood cells, for comparison with activity against a eukaryotic microbe such as *C. albicans*. We focused on sequences containing *trans*-2-aminocyclohexanecarboxylic acid (ACHC) residues, which have a much higher 14-helical propensity than do β-amino acid residues bearing a side chain adjacent to the nitrogen atom (β^3-residues).[11]



We were pleased to find significant antifungal activity for 14-helical β-peptides, given the lack of activity observed for the host defense α-peptides (Table 1). The decamer β^3Tyr-(ACHC-β^3Val-

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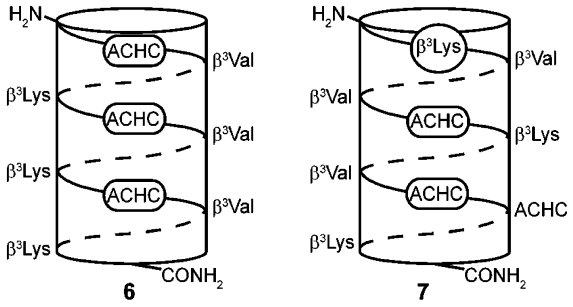

Figure 1. Three-dimensional representation of globally amphiphilic and scrambled 14-helical β-peptides **6** and **7**.

β^3Lys)$_3$ (**1**) displayed a reasonably low MIC (16 μg/mL) and only a moderate degree of hemolysis at the MIC (21%). The 14-helix has approximately three residues per turn; therefore, the triad repeat in this deca-β-peptide should generate a globally amphiphilic structure in which the hydrophobic ACHC and β^3Val residues are clustered on one side of the helix and the cationic β^3Lys residues are clustered on the other side. The N-terminal β^3Tyr residue was included to aid absorbance-based concentration determination.

β-Peptides **2**−**5**, analogues of **1**, were evaluated to elucidate relationships among sequence, folding, and antifungal and hemolytic activities (Table 1). Circular dichroism (CD) in aqueous buffer[12] indicates that each of the ACHC-containing β-peptides has a substantial 14-helical population (**5** > **1** > **3** > **2**), while, as expected, little or no 14-helicity is evident for **4**, which contains exclusively β^3-residues.[11] The fact that **4** displays substantially weaker antifungal activity than do analogues **1**−**3** and **5** suggests that 14-helical folding is important for this activity. However, neither antifungal nor hemolytic activity is directly correlated with the order of 14-helical folding indicated by the CD data, which suggests that the biological activities are determined by an interplay among conformational propensity and other physicochemical properties such as net hydrophobicity. The decamer length appears to be optimal, since no antifungal activity could be detected when **1** was truncated or extended by one ACHC-β^3Val-β^3Lys triad (not shown).

Previous work with β-peptides closely related to **1** revealed that removal of the N-terminal β^3Tyr residue decreases hemolytic activity without diminishing antibacterial activity.[4g] We found a similar effect in terms of antifungal activity and selectivity, as indicated by the behavior of (ACHC-β^3Val-β^3Lys)$_3$ (**6**): the average MIC is indistinguishable from that of β^3Tyr-containing variant **1**, but without the β^3Tyr only 5% hemolysis occurs at the MIC.

The importance of 14-helical folding for antifungal activity is shown by the dramatic contrast between **6** and sequence isomer β^3Lys-β^3Val-β^3Val-ACHC-β^3Lys-β^3Val-ACHC-ACHC-β^3Lys (**7**). The 14-helical conformation available to **7** is not globally amphiphilic, because the cationic β^3Lys residues are distributed around the helix circumference rather than aligned along one side (Figure 1). We have previously shown that analogous "scrambled" β-peptide sequence isomers are completely inactive against bacteria,[4f,g] which is consistent with the lack of antifungal activity reported here for scrambled β-peptide **7**. β-Peptide **5** has been shown to self-associate,[13] and **1**−**4** and **6** may display similar behavior; self-association of α-peptides can influence antimicrobial activity.[3]

Our results show, for the first time, that unnatural foldamers can display significant antifungal activity. Although the best β-peptides described here do not match the most potent small molecules in terms of in vitro activity (e.g., amphotericin B, for which MIC = 0.7 μg/mL under our assay conditions), our findings are significant because they identify a new class of antifungal agents that, by virtue of modular structure, can be easily modified in pursuit of improved activity. The contrast between the antibacterial and antifungal arenas is striking. A wide variety of designed β-peptides, including some identical or closely related to those discussed here, display antibacterial activities rivaling those of host-defense α-peptides, but no β-peptide has significantly exceeded α-peptide performance.[4] Here we have demonstrated antifungal activity of β-peptides under conditions (pH 7, physiological ionic strength) that do not support host-defense amphiphilic α-peptide activity.

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Supporting Information Available: β-Peptide synthesis, CD analysis, and biological assay protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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